

Coacervate Laboratory: How are protobiotic molecular assemblies formed? What variables effect the formation of these assemblies?

Background:

As we ask ourselves about what life is we also want to know the conditions under which life can form and flourish. Exobiologists are especially interested in how life forms and what conditions are necessary as they search for life on other planets.

Researchers, including Oparin and Haldane (1930's), explored the possible patterns of formation of the very first pre-cellular entities from abiotic materials. A whole series of such "protobionts" have been repeatedly generated by various experiments the past 80+ years. Protobionts are types of pre-cellular structures that form abiotically from the interaction of various organic molecules. Many of these protobiont structures possess some biological properties.

Urey and Miller (1953) generated coacervates, which are droplets that self-assemble when various combinations of abiotically made organic molecules such as nucleic acids, polypeptides, and polysaccharides are shaken together in a closed environment with external energy supplied. If enzymes are added to the solution, the coacervates will incorporate them and are able to use them to perform reactions, make products, and release the products.

In this lab we will use Gelatin (a protein) and Gum Arabic (a simple carbohydrate) to create coacervates. At first, we will be manipulating the pH of the solution to test its effect on the formation and duration of our coacervates. pH is a measure of the acidity of a solution. pHs under 7 indicate an acid, a pH of 7 is neutral and pHs above 7 are basic. After we establish the best pH range for coacervates, you will design a text of another variable. Examples include: concentration of Gum Arabic or gelatin, ratio of Gum Arabic to Gelatin, amount of shaking, using a different acid or base instead of HCl.

Materials:

1% gelatin solution, droppers	microscope slides
1% gum arabic solution	coverslips
pH papers	0.1M HCl
glass stirring rod	test tubes
microscopes	test tube rack

Safety:

Wear goggles! Coverslips and microscope slides can have sharp edges-use caution when handling and washing. Use caution with the acid HCl (hydrochloric acid) even though we are using a very dilute solution you must rinse it off immediately if you spill any on the lab table or on yourself.

Procedure:

1. Before you begin: Read the entire lab. Prepare your lab notebook- for this lab you will need to create a chemical table for gum Arabic, gelatin, and HCl.

Laboratory 1
Hrs Biology
Stone

Part A: With your lab partner.

2. Measure 5mL of 1% gelatin solution. Pour it into a clean test tube. Record observations about the color and clarity of this solution.
3. Measure 3 mL of 1% gum arabic solution into a second clean test tube. Record observations about the color and clarity of this solution.
4. Record the pH of each solution by dipping the glass stirring rod into the solution and then gently touching the pH paper with the stirring rod. Compare any color change on the pH paper to the label and record the pH estimate.
5. Make a wet mount of each mixture. Observe under low and high power and record your observations.
6. Pour the gum Arabic solution into the gelatin solution. Mix gently by inversion as demonstrated by Mrs. Stone. Repeat steps 4 and 5 for this mixture.

Part B: With your lab partner.

7. Stir one drop of 0.1M HCl solution into the mixture. Record the mixture's pH.
8. Repeat step 5 for the HCl mixture.
9. Repeat steps 7 and 8 until you see coacervates, clumps that look like cells. Record the mixture's pH and describe the appearance of the coacervates. Count the number of coacervates you see in three different fields of view on high power. Average and record for each pH in which you see coacervates.
10. Apply Methylene blue stain as demonstrated by Mrs. Stone. Methylene blue is used to stain the nuclei of cells. After you have applied the stain observe the slide again under low and high power and record your results.
11. Repeat steps 7 and 8 until the coacervates disappear.

Part C: As a lab table.

12. Decide on a variable (you may test more than one variable) that you want to test and write a procedure for how you will perform your test. Mrs. Stone must approve this before you can begin.
13. After you have completed your test you will present your findings to the class as a google presentation. Your presentation must include a description of your test, pictures, a graph, and a conclusion regarding the effect of your variable on the formation of coacervates. Electronically share your presentation with the other members of the class.

Analysis and Conclusions: (Answer in complete sentences)

1. Why are steps 4 and 5 important?
2. Around what pH did you observe coacervates? Why do you think the formed and then went away as you continued to add HCl?
3. Make an excel graph of the average coacervates formed at a given pH.
4. What about living things do coacervates resemble?
5. What did your lab table test for part C and what did you find?
6. Read the other presentations from your class and then write an overall conclusion for the lab that addresses the factors that affect coacervate formation. You should also write about any difficulties you encountered in the lab and what you would change if you were going to perform it again.

Laboratory 1
Hrs Biology
Stone

Teacher Notes:

Materials per class:

Microscopes (1/pair)
Computers (6)
Temp probes or Thermometers (6-12)
Slides (minimum of 12)
Coverslips (minimum of 12)
Lens Paper
Kimwipes
Gum Arabic (1% solution) min 250mL/class with 12 lab groups
Gelatin (1% solution) min 250mL/class with 12 lab groups
0.10 M HCL in 12 dropper bottles
Hydrion or pH test paper 6-12
Test tubes and test tube racks
Glass stirring rods

Demonstrate:

1. Preparation of chemical table and where to find information.
2. Preparation of wet-mount slides
3. How to stain slides.
4. How to mix by inversion.